Expression of nuclear receptors and glucose metabolic pathway proteins in sebaceous carcinoma: Androgen receptor and monocarboxylate transporter 1 have a key role in disease progression

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Abstract. Standard systemic treatments are not consistently effective for treating unresectable or advanced sebaceous carcinoma (SC). The present study investigated the pathogenic roles of nuclear receptors (NRs), glucose metabolic dysregulation and immune checkpoint proteins in SC as prognostic markers or therapeutic targets. Patients with pathologically confirmed SC between January 2002 and December 2019 at three university hospitals in South Korea were included in the present study. Immunohistochemistry was performed on paraffin-embedded tumor tissues for glucocorticoid receptors (GR), androgen receptors (AR), estrogen receptors (ER), progesterone receptors (PR), glucose transporter 1 (GLUT1), monocarboxylate transporters (MCT1 and MCT4), CD147, phosphorylated adenosine monophosphate-activated protein kinase (pAMPK) and the immune checkpoint protein, programmed cell death-ligand 1 (PD-L1). The results were semi-quantitatively assessed and the associations of these proteins with various clinicopathological parameters were determined. A total of 39 cases of SC comprising 19 periocular and 20 extraocular tumors were enrolled. NRs were frequently detected in the tumor nuclei, with GR having the highest frequency (89.7%), followed by AR, ER (both 51.3%) and PR (41.0%). Regarding glucose metabolism, CD147, GLUT1 and MCT1 were highly expressed at 100, 89.7 and 87.2%, respectively, whereas MCT4 and pAMPK expression levels were relatively low at 38.5 and 35.9%, respectively. Membranous expression of PD-L1 was detected in five cases (12.8%), four of which were extraocular. In the multivariate analysis, advanced stage, low AR positivity and high MCT1 expression were independent poor prognostic factors for metastasis-free survival (all $P<0.05$). The present results suggested that hormonal and metabolic dysregulation may be associated with the pathogenesis of SC, and that AR and MCT1 in particular may serve as prognostic indicators and potential therapeutic targets. Additionally, ~10% of SC cases exhibited PD-L1 expression within the druggable range, and these patients are expected to benefit from treatment with immune checkpoint inhibitors.

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Introduction

Over the past few decades, rapid advances in genomic technology and the accumulation of knowledge in cancer biology

have shifted the paradigm of cancer chemotherapy from conventional cytotoxic small-molecule drugs to targeted and personalized approaches (1). Sebaceous carcinoma (SC) is a rare but aggressive malignancy arising from the adnexal epithelium of the sebaceous glands and is primarily treated surgically; however, the possibility of disease recurrence or metastasis after resection is higher than that of other eyelid malignancies. As SC frequently occurs on the eyelid, extensive resection is often difficult for functional or cosmetic reasons. However, there are no standardized protocols or highly effective agents for the treatment of patients with advanced SC (2). The pathogenesis of SC remains poorly understood, and studies investigating therapeutic targets for SC are limited compared to those of recent therapeutic breakthroughs for other cutaneous malignancies (3-6). Recently, several researchers, including our group, have investigated the genomic landscape of SC (7) and revealed candidates for potential targetable alterations, such as PIK3CA, EGFR, and BRAF. However, because these mutations are low in frequency and are not closely associated with clinical outcomes, there is still a need to identify more universal targets, such as hormonal receptors, in breast cancer.

Sebocytes are metabolically active cells that release numerous cytokines and chemokines under the influence of hormones to maintain epidermal barrier and immune functions (8,9). Steroid hormone receptors, such as glucocorticoid receptors (GR), androgen receptors (AR), estrogen receptors (ER), and progesterone receptors (PR), are nuclear transcription factors that participate in cellular differentiation and metabolic processes (10) and are pathogenetically linked to solid tumors, most representatively breast and prostate cancers (11-13). Among the NRs, only the AR has been studied for its expression and relationship with SC (14,15). For diagnostic purposes, AR is a sensitive marker of sebaceous differentiation and is particularly useful for identifying poorly differentiated sebaceous carcinoma (16,17). However, the clinical significance of AR expression in SC has not been clearly established, and its relationship with other NRs remains unknown.

One of the most important functions of NRs is the regulation of metabolism and inflammation, both of which are involved in cancer pathogenesis. As cancer cells require more energy than normal cells do, alterations in glucose metabolism, called the Warburg effect or anaerobic glycolysis, occur in cancer cells, resulting in excessive accumulation of lactate and acidification of the extracellular pH in the tumor microenvironment (18-21). These environmental changes caused by NRs are associated with the aggressive biological behavior of cancer cells by enhancing metastasis, angiogenesis, and immunosuppression (22). Additionally, a recent study has shown that lactate promotes the expression of programmed cell death-1 (PD-1) in regulatory T cells in the tumor microenvironment (23).

Given this background, we hypothesized that altered NRs activity is associated with changes in glucose metabolism and the immune microenvironment of SC. We investigated the expression of four NRs and glucose metabolic pathway proteins, including glucose transporter 1 (GLUT1), monocarboxylate transporters (MCT1 and MCT4), CD147, phosphorylated adenosine monophosphate-activated protein kinase (pAMPK), and PD-L1 and correlated them with various clinicopathological parameters. We sought to determine their pathogenic role and clinical significance in SC.

Materials and methods

Patients. Patients diagnosed and treated for SC at Seoul National University Hospital (Seoul, South Korea), Seoul Metropolitan Government-Seoul National University Boramae Medical Center (Seoul, South Korea), and Seoul National University Bundang Hospital (Seongnam, South Korea) between January 2002 and December 2019 were included in this study. Clinical data were collected from medical records, and pathological diagnoses were confirmed by an experienced pathologist. Demographic information; histopathological features; anatomical location; treatment details; outcomes, such as local recurrence and nodal or distant metastases; and survival time were reviewed. All tumors were restaged according to the American Joint Committee on Cancer (AJCC) staging system, 8th edition.

This study was approved by the Institutional Review Board of Seoul National University Hospital (H-1905-059-1032). This study complied with the principles of the Declaration of Helsinki.

Immunohistochemistry. Immunohistochemistry (IHC) was performed on 4-μm-thick serial sections of formalin-fixed, paraffin-embedded tissue samples from patients with SC using an automated staining platform (BenchMark ULTRA, Ventana, Tucson, AZ). The test items included the GR (cat. no. 3660, Cell Signaling, Danvers, MA, USA), ER (cat. no. M7047, Dako, Carpinteria, CA, USA), PR (cat. no. M3569, Dako), AR (cat. no. MA5-13426, Thermo Fisher Scientific, Carlsbad, CA, USA), MCT1 (cat. no. SC-365501, Santa Cruz Biotechnology, Dallas, TX, USA), MCT4 (cat. no. SC-376140, Santa Cruz Biotechnology), GLUT1 (cat. no. ab15309, Abcam, Cambridge, UK), CD147 (cat. no. MA5-29060, Thermo Fisher Scientific), pAMPK (cat. no. 2535, Cell Signaling), and PD-L1 (cat. no. 741-4905, SP263, Ventana). A standardized protocol was used according to the manufacturer's recommendations. Dried sections were deparaffinized in xylene and rehydrated using a series of graded ethanol solutions (95, 85, 70, and 55%) at room temperature for 10 min. Heat-induced epitope retrieval was performed in a pressure cooker at 95˚C for 2 min using 0.01 M citrate buffer. Slides were incubated overnight at 4˚C for all the primary antibodies and washed with phosphate-buffered saline four times. The UltraView Universal DAB Detection Kit (cat. no. 760-500, Ventana) was used to visualize the primary antibodies with 3,3'-diaminobenzidine tetrahydrochloride chromogen. An experienced pathologist (JEK) performed semi-quantitative interpretation using a BX51 light microscope (magnification, x200 and x400) (Olympus Corporation, Tokyo, Japan) blinded to the clinical data. For GR, ER, and PR, the result was considered positive if ≥1% of the tumor cell nuclei were immunoreactive. However, considering that AR is consistently found in normal sebaceous glands, it was interpreted as high or low on the basis of 10%. The PD-L1 test result was considered positive if ≥1% of tumor cells showed membrane staining, according to the guidelines of the Ventana PD-L1 SP263 assay approved for non-small cell lung cancer (https://diagnostics.roche.com/global/en/products/lab/pd-l1-sp263-ce-ivd-us-export-ventana-rtd001234.html). For the metabolic markers, the H-score was generated by multiplying the intensity (0-3+) by the percentage of positive

Table I. Demographic data of 39 patients with sebaceous carcinoma.

Clinicopathologic features	Value
Mean ± SD age, years (range)	69.5±15.5 (26-97)
Sex, n (%)	
Male	17 (43.6)
Female	22 (56.4)
Mean ± SD follow-up, months (range)	51.4±46.5 (5-258)
Primary site, n (%)	
Periocular	19 (48.7)
Extraocular	20 (51.3)
Initial stage, n (%)	
Localized	32 (82.1)
Advanced (lymph node involvement or distant metastasis)	7 (17.9)
T category, n (%)	
T1	17 (43.6)
T2	15 (38.5)
T3	2 (5.1)
T4	5 (12.8)
Treatment outcome, n (%)	
No recurrence	23 (59.0)
Local recurrence	5 (12.8)
Nodal or distant metastasis	11 (28.2)

SD, standard deviation.

Table II. Correlation between the expression of nuclear receptors, PD-L1, and glucose metabolic markers in sebaceous carcinoma using nonparametric Spearman's rank correlation analysis.

	Spearman's rank correlation coefficients				
Marker	MCT1	MCT4	GLUT1	CD147	pAMPK
GR	-0.002	-0.264	-0.117	0.047	0.388[a]
AR	0.048	-0.154	0.094	0.204	-0.137
ER	-0.017	0.023	0.252	-0.228	-0.285
PR	0.327[a]	-0.158	-0.503[a]	-0.052	0.538[a]
PD-L1	-0.003	-0.170	-0.231	0.058	0.118

[a]P<0.05. AR, androgen receptor; ER, estrogen receptor; GLUT, glucose transporter; GR, glucocorticoid receptor; MCT, monocarboxylate transporter; pAMPK, phosphorylated-AMP-activated protein kinase; PD-L1, programmed cell death ligand1; PR, progesterone receptor.

tumor cells, with scores ranging from 0 to 300; an H-score of 10 or higher was considered positive (https://diagnostics.roche.com/global/en/products/lab/pd-l1-sp263-ce-ivd-us-export-ventana-rtd001234.html).

Statistical analyses. Fisher's exact and χ^2 tests were performed to determine the differences or associations among categorical variables. Differences among the IHC expression profiles and clinical data of the patients were examined using non-parametric Mann-Whitney U tests. Correlations between the expression of NRs, PD-L1 and glucose metabolic markers were analyzed using the nonparametric Spearman correlation test. Univariate Kaplan-Meier analysis with a log-rank test was used to evaluate post-operative metastasis-free survival between the groups based on pathologic parameters. Cox proportional hazards regression was used to identify the parameters associated with metastasis-free survival. Statistical analyses were performed using the IBM SPSS software version 25 (IBM, Armonk, NY, USA). All P-values reported were two-sided, and P<0.05 was considered to indicate a statistically significant difference.

Results

Patient demographics and clinical features. Table I lists the baseline demographic characteristics of patients. A total of 39 cases of SC were included, of which 19 were periocular and 20 were extraocular tumors. Based on the AJCC 8th edition criteria, 32 (82.1%) patients had tumors at the T2 level or lower, and seven (17.9%) patients had tumors at the T3 level or higher. Lymph node involvement or distant metastasis was detected in seven (17.9%) patients at the time of treatment. During the follow-up period (mean: 51.4 months; range, 5-258 months), five (12.8%) patients had local recurrence, and 11 (28.2%) patients presented with nodal or distant metastases.

Immunohistochemistry results. Representative images of positive immunoreactivity for NRs and PD-L1 in SC are shown in Fig. 1. Glucose metabolic pathway-related proteins are shown in Fig. 2. In all 39 SC cases, the NR positivity rate was 35 (89.7%) for GR, 20 (51.3%) for AR and ER, and 16 (41.0%) for PR. Membranous expression of PD-L1 was found in five cases (12.8%). Regarding glucose metabolism, CD147, GLUT1, and MCT1 were positively and highly expressed in 39 (100%; median H-score:300), 35 (87.2%; median H-score: 240), and 34 (87.2%; median H-score: 50) patients, respectively. However, MCT4 and pAMPK cells showed low positivity rates and relatively low expression levels (38.5%, median H-score: 0 and 35.9%, median H-score: 0, respectively).

To investigate the correlation between each IHC marker, a nonparametric Spearman's rank correlation analysis was performed. PR expression positively correlated with MCT1 and pAMPK (P=0.042 and P=0.001, respectively), but negatively correlated with GLUT1 expression (P=0.001). GR levels were positively correlated with pAMPK levels (P=0.015). However, the expression of AR, ER, and PD-L1 was not significantly associated with that of the glucose metabolic markers (Table II).

We performed a stratified analysis to explore the correlations between NR, PD-L1, and glucose metabolic markers in the periocular and extraocular SC groups (Tables SI and SII). In the periocular SC group, a significant negative correlation was observed between AR and pAMPK (P=0.038) and between PR and GLUT1 (P=0.002). In the extraocular SC group, PR expression was positively correlated with MCT1 and pAMPK levels (P=0.010 and P=0.001, respectively). Additionally, a

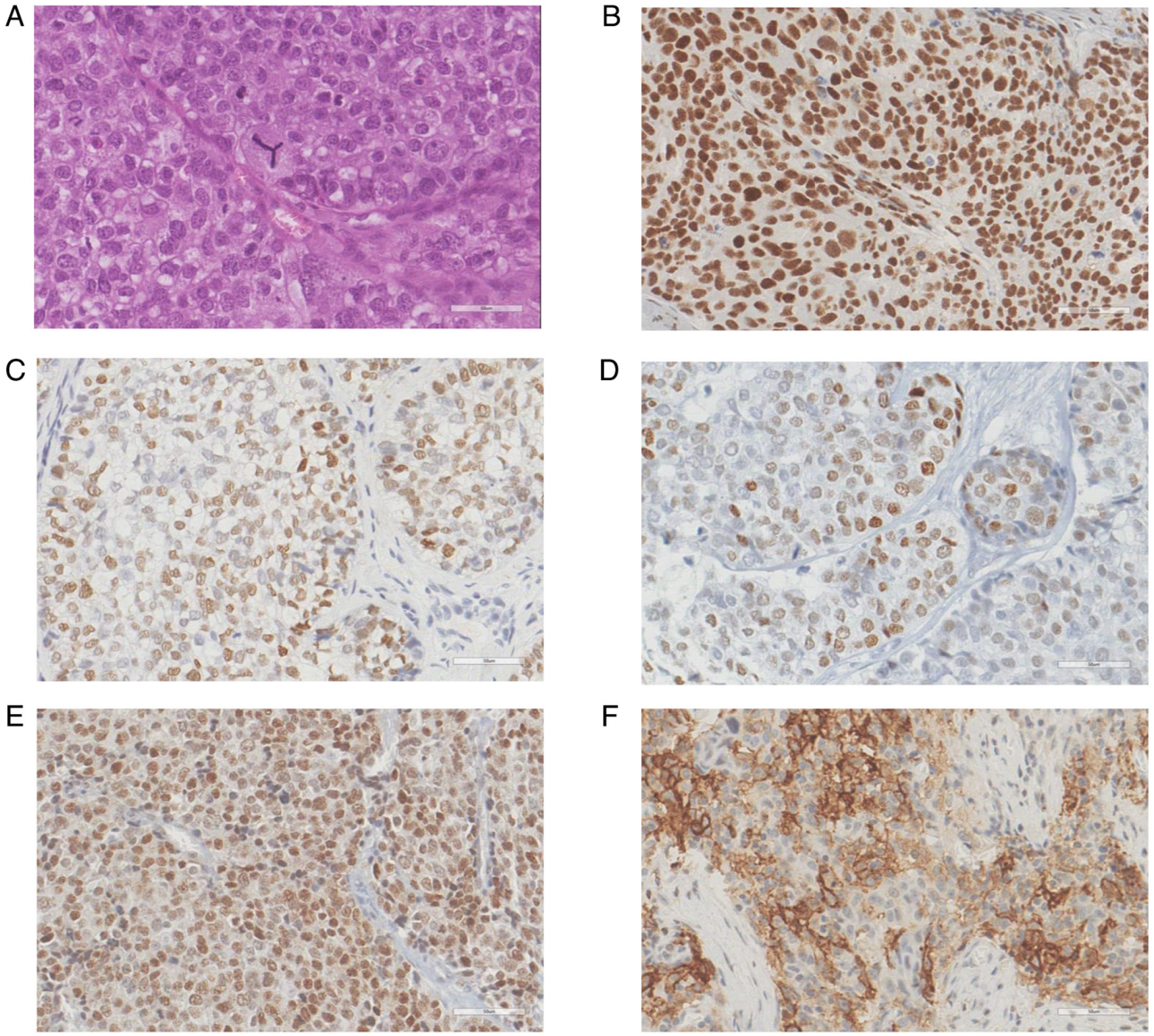

Figure 1. Expression of nuclear receptors and PD-L1 in SC (all images at magnification, x400). The transparent bar in the bottom-right corner represents the scale bar, indicating 50 μm. (A) Histopathologically, SC exhibits solid sheets of atypical cells with vacuolated cytoplasm (Hematoxylin-Eosin). Representative figures of positive immunoreactivity for (B) glucocorticoid receptors, (C) androgen receptors, (D) estrogen receptors, (E) progesterone receptors, and (F) PD-L1. PD-L1, programmed cell death-ligand; SC, sebaceous carcinoma.

significant positive correlation was observed between ER and GLUT1 expression (P=0.030). These findings indicate that the molecular interactions between these biomarkers may differ depending on the tumor origin, underscoring the potential influence of anatomical sites on the biological behavior of SC.

Clinicopathologic correlation. We compared the clinical features and IHC results between the 19 periocular and 20 extraocular SC groups (Table III). No significant differences were found in the clinical characteristics or protein expression levels between the two groups, except for the extent of the primary tumors (higher T stage in periocular tumors). Notably, four of the five cases showing PD-L1 expression were extraocular. However, no significant relationships were identified between PD-L1 expression and clinical variables, such as tumor origin, T grade, disease stage, or clinical outcome.

The clinicopathological features and IHC results according to the disease progression (postoperative metastasis) status are shown in Table IV. Significant differences were found in the T category and stage, with the disease progression group exhibiting higher T and advanced-stage tumors (P=0.012 and P=0.001, respectively). Among the NRs, AR was the only one whose expression was significantly higher in the group without disease progression than in that with disease progression (P=0.005). No significant differences were observed between the two groups in the expression levels of glucose metabolism markers.

Additionally, we stratified the patients into periocular and extraocular groups to assess differences in the expression of various markers between those with and without disease progression (Tables SIII and SIV). This analysis mirrored the trends observed in the entire cohort (Table IV), particularly regarding the association between low AR expression and

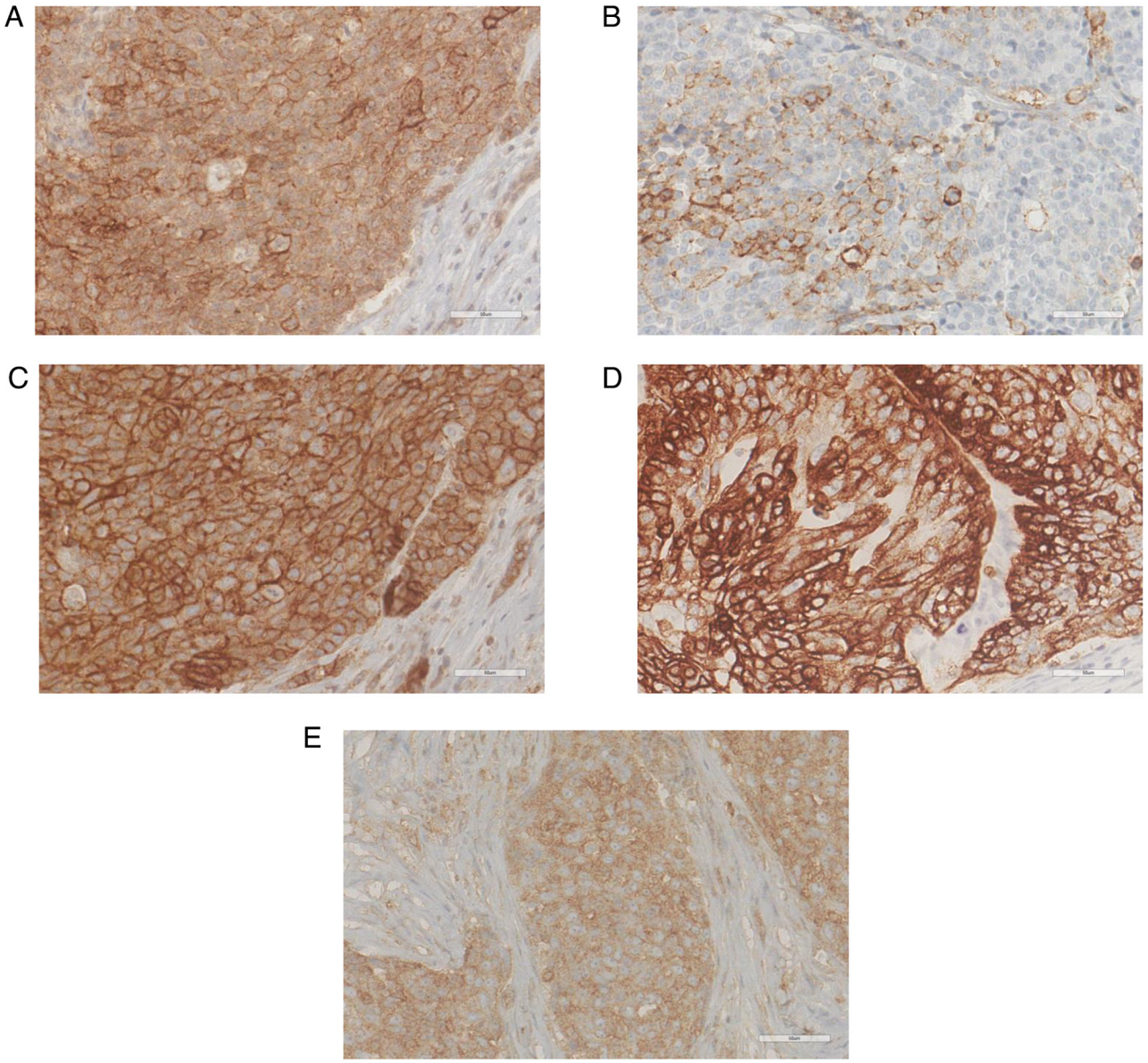

Figure 2. Expression of glucose metabolic pathway-related proteins in sebaceous carcinoma (all images at magnification, x400). The transparent bar in the bottom-right corner represents the scale bar, indicating 50 μm. (A) MCT1, (C) CD147 and (D) glucose transporter 1 were diffusely expressed in most of the cases. However, (B) MCT4 and (E) phosphorylated adenosine monophosphate-activated protein kinase were expressed in a subset of the cases. MCT, monocarboxylate transporter.

disease progression. Specifically, low AR expression was observed in 87.5 and 75.0% of patients in the periocular and extraocular groups, respectively. However, the statistical significance of these findings could not be established.

In univariate survival analysis, patients who exhibited high AR expression had significantly longer metastasis-free survival compared to those who did not (P=0.006) (Fig. 3A), whereas other NRs did not affect patient outcomes. High MCT1 expression was negatively associated with patient survival (P=0.019; Fig. 3B). Expression of other glucose metabolic markers or PD-L1 was not associated with patient prognosis.

Multivariate analysis revealed that the advanced stage of the initial tumor presentation, low AR, and high MCT1 expression levels were independent poor prognostic factors for metastasis-free survival (P=0.039, P=0.034, and P=0.021, respectively; Table V).

Discussion

This study comprehensively investigated the prognostic and therapeutic significance of NRs, glucose metabolic alterations, and PD-L1 expression in SC. Most SC cases in our cohort exhibited relatively high levels of all four NR expressions and significant levels of glucose metabolism-related proteins. Specifically, this study highlighted that low AR and high MCT1 expression levels were independent poor prognostic factors.

Research on the role of NRs, which are important regulators of various transcriptional pathways involved in the development or progression of cancer, as diagnostic markers and targets for hormonal therapy has recently attracted increasing attention (24). Most published studies investigating NRs in SC have focused primarily on the expression status of

Table III. Comparison of the clinicopathologic findings in sebaceous carcinoma according to the primary site.

Characteristic	Periocular (n=19)	Extraocular (n=20)	P-value[c]
Sex, n (%)			
Male	6 (31.6)	11 (55.0)	0.200
Female	13 (68.4)	9 (45.0)	
Mean ± SD age, years	72.8±15.0	66.4±15.7	0.122
T category, n (%)			
T2 or lesser	13 (68.4)	19 (95.0)	0.044[d]
T3 or higher	6 (31.6)	1 (5.0)	
Initial stage, n (%)			
Localized	15 (78.9)	17 (85.0)	0.695
Advanced[a]	4 (21.1)	3 (15.0)	
Disease progression, n (%)[b]			
Absent	11 (57.9)	16 (80.0)	0.176
Present	8 (42.1)	4 (20.0)	
GR, n (%)			
Negative	1 (5.3)	3 (15.0)	0.605
Positive	18 (94.7)	17 (85.0)	
AR, n (%)			
Low	12 (63.2)	9 (45.0)	0.341
High	7 (36.8)	11 (55.0)	
ER, n (%)			
Negative	8 (42.1)	11 (55.0)	0.421
Positive	11 (57.9)	9 (45.0)	
PR, n (%)			
Negative	10 (52.6)	13 (65.0)	0.433
Positive	9 (47.4)	7 (35.0)	
PD-L1, n (%)			
Negative	18 (94.7)	16 (80.0)	0.342
Positive	1 (5.3)	4 (20.0)	
MCT1, n (%)			
Low	18 (94.7)	17 (85.0)	0.605
High	1 (5.3)	3 (15.0)	
MCT4, n (%)			
Low	12 (63.2)	12 (60.0)	>0.999
High	7 (36.8)	8 (40.0)	
GLUT1, n (%)			
Low	7 (36.8)	11 (65.0)	0.341
High	12 (63.2)	9 (35.0)	
CD147, n (%)			
Low	7 (36.8)	9 (35.0)	0.748
High	12 (63.2)	11 (65.0)	
pAMPK, n (%)			
Low	16 (84.2)	13 (65.0)	0.273
High	3 (15.8)	7 (35.0)	

[a]Lymph node involvement or distant metastasis. [b]Disease progression: Postoperative lymph node involvement or distant metastasis. [c]χ^2 test or Fisher's exact test were used to compare categorical variables; the comparison of mean values was performed using the Mann-Whitney U test. [d]P<0.05. AR, androgen receptor; ER, estrogen receptor; GLUT, glucose transporter; GR, glucocorticoid receptor; MCT, monocarboxylate transporter; pAMPK, phosphorylated-AMP-activated protein kinase; PD-L1, programmed cell death ligand1; PR, progesterone receptor; SD, standard deviation.

Table IV. Comparison of clinicopathologic findings based on disease progression.

Characteristic	Sebaceous carcinoma without progression[a] (n=28)	Sebaceous carcinoma with progression (n=11)	P-value[b]
Sex, n (%)			
Male	11 (39.3)	6 (54.5)	0.387
Female	17 (60.7)	5 (45.5)	
Mean ± SD age, years (range)	70.7±15.7 (26-97)	66.6±15.2 (36-89)	0.357
Mean ± SD follow-up, months (range)	50.3±33.4 (5-136)	54.2±72.2 (8-258)	0.318
Primary site, n (%)			
Periocular	12 (42.9)	7 (63.6)	0.301
Extraocular	16 (57.1)	4 (51.3)	
T category, n (%)			
T2 or lesser	26 (92.9)	26 (54.5)	0.012[c]
T3 or higher	2 (7.1)	2 (45.5)	
Initial stage, n (%)			
Localized	27 (96.4)	5 (45.5)	0.001[c]
Advanced (lymph node involvement or distant metastasis)	1 (3.6)	6 (54.5)	
GR, n (%)			
Negative	2 (7.1)	2 (18.2)	0.562
Positive	26 (92.9)	9 (81.8)	
AR, n (%)			
Low	11 (39.3)	10 (90.9)	0.005[c]
High	17 (60.7)	1 (9.1)	
ER, n (%)			
Negative	12 (42.9)	7 (63.6)	0.301
Positive	16 (57.1)	4 (36.4)	
PR, n (%)			
Negative	17 (60.7)	6 (54.5)	0.725
Positive	11 (39.3)	5 (45.5	
PD-L1, n (%)			
Negative	24 (85.7)	10 (90.9)	>0.999
Positive	4 (14.3)	1 (9.1)	
MCT1, n (%)			
Low	26 (92.9)	9 (81.8)	0.562
High	2 (7.1)	2 (18.2)	
MCT4, n (%)			
Low	15 (53.6)	9 (81.8)	0.150
High	13 (46.4)	2 (18.2)	
GLUT1, n (%)			
Low	14 (50)	4 (36.4)	0.497
High	14 (50)	7 (63.6)	
CD147, n (%)			
Low	13 (46.4)	3 (27.3)	0.471
High	15 (53.6)	8 (72.7)	
pAMPK, n (%)			
Low	22 (78.6)	7 (63.6)	0.424
High	6 (21.4)	4 (36.4)	

[a]Disease progression: Postoperative lymph node involvement or distant metastasis. [b]χ^2 test or Fisher's exact test were used to compare categorical variables; the comparison of mean values was performed using the Mann-Whitney U test. [c]P<0.05. AR, androgen receptor; ER, estrogen receptor; GLUT, glucose transporter; GR, glucocorticoid receptor; MCT, monocarboxylate transporter; pAMPK, phosphorylated-AMP-activated protein kinase; PD-L1, programmed cell death ligand1; PR, progesterone receptor; SD, standard deviation.

Table V. Multivariable Cox proportional regression analysis of metastasis-free survival.

Characteristic	Hazard ratio	95% confidence interval		P-value
		Lower	Upper	
Age	1.04	0.99	1.09	0.168
Primary site (Periocular: Extraocular)	0.24	0.02	2.72	0.246
T category (T2 or lesser: T3 or higher)	0.20	0.02	2.27	0.194
Initial stage (Localized: Advanced[a])	4.15	1.08	15.98	0.039[b]
GR	0.07	0.00	1.44	0.085
AR	0.04	0.00	0.78	0.034[b]
PD-L1	7.27	0.20	26.67	0.281
MCT1	41.90	1.77	99.07	0.021[b]
MCT4	0.02	0.00	1.10	0.056

[a]Lymph node involvement or distant metastasis. [b]P<0.05. AR, androgen receptor; ER, estrogen receptor; GR, glucocorticoid receptor; MCT, monocarboxylate transporter; PD-L1, programmed cell death ligand 1; PR, progesterone receptor.

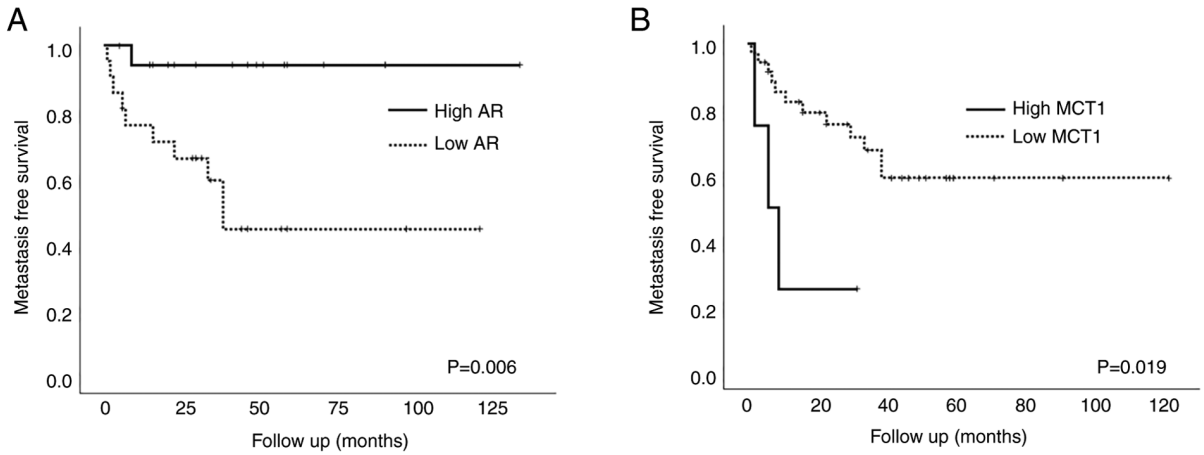

Figure 3. Kaplan-Meier plots of sebaceous carcinoma on risk factors for the progression of metastasis. (A) Patients with AR expression had significantly longer metastasis-free survival as determined via univariable analysis performed using log-rank tests (P=0.006). (B) High MCT1 expression was negatively associated with superior survival (P=0.019). AR, androgen receptors; MCT1, monocarboxylate transporter 1.

AR, a sensitive marker of sebaceous differentiation (14,16,25). Findings regarding the significance of AR as a prognostic marker for SC are conflicting (25-27). The current study revealed that AR expression is a potential prognostic indicator and provides a new perspective for therapeutic interventions. Breast and prostate cancers are the most common types of cancers treated with anti-hormonal agents. AR is an emerging and promising therapeutic target in a subset of triple-negative breast cancer (TNBC), an aggressive subtype that lacks ER, PR, or human epidermal growth factor receptor 2 (HER-2) (28). Recent studies have shown that patients with AR-positive TNBC may benefit from treatment with AR inhibitors, such as bicalutamide, enzalutamide, and abiraterone, with tolerable toxicity (28-32). Because AR expression indicates less chemosensitivity and a favorable prognosis in TNBC, the introduction of AR inhibitors may lead to a change in treatment modalities. However, the role of AR in SC is expected to be different from that in breast cancer because AR is activated in normal sebocytes and downregulation of AR indicates a lack of differentiation or even dedifferentiation. However, for some patients with high AR expression who fail initial treatment, AR inhibitors may be an alternative.

To the best of our knowledge, this is the first study examining GR expression in SC. The function of the GR in various cancer cells is well understood, both experimentally and clinically. Latorre *et al* (33) demonstrated that GR deficiency accelerates epidermal tumor growth and skin cancer growth in knockout mouse models. In hormone-dependent solid tumors, GR performs diverse functions that regulate cellular differentiation, apoptosis, and proliferation (34-42). GR expression has been demonstrated in various types of cancer cells and serves as a favorable prognostic indicator and predictor of anti-GR agents (40). The majority of SC cases in our study showed relatively high levels of GR expression, suggesting a pivotal role for GR in the pathogenesis of SC. Only four of 39 (10.3%) patients had GR-negative tumors, but two (50%) developed distant metastases during follow-up. Although statistical significance regarding patient survival could not be

confirmed owing to the small number of GR-negative cases, the loss of GR appears to be closely related to disease progression or invasive potential. Furthermore, GR and AR share a canonical hormone-responsive element, and these two NRs regulate overlapping sets of genes. Therefore, it is unclear whether the GR plays an independent or AR-dependent role in SC pathogenesis (43,44).

Changes in the metabolic environment caused by the upregulation of NRs are common during cancer progression (18-21). Glucose is transported by membrane-associated GLUT family proteins that are carefully controlled under normal circumstances; however, increased glucose uptake and the switch to aerobic glycolysis, known as the Warburg effect, are prominent metabolic alterations observed in most types of cancer (18-21). This results in the rapid generation of ATP along with increased glucose uptake and lactate formation. Furthermore, to facilitate lactate transport, the activities of MCT1 and MCT4, along with those of CD147, a chaperone for both proteins, are increased in cancer cells. More specifically, MCT1 is responsible for accumulating lactate in cells, whereas MCT4 contributes to the transporting lactate out of the cells (45). Although MCT1 and MCT4 appear to act in opposite directions, their dysregulation occurs in many types of cancer, resulting in the activation of both proteins (46). These metabolic markers may not only be poor prognostic factors (47-54) but may also be potential therapeutic targets (55-58). To date, studies on metabolic changes in SC are limited. Only one study has proposed GLUT1 as a diagnostic marker for differentiating SC from benign sebaceous lesions (59). In our study, glucose metabolic markers were expressed to varying degrees across the cases, with the most frequently expressed being GLUT1 and pAMPK, suggesting that these two indicators may also be used for diagnostic purposes. However, the only metabolic indicator related to patient outcome was MCT1, although its expression rate was not high. Our results provide evidence that MCT1 plays a pivotal role in tumor progression and that metabolic transporters could serve as potential therapeutic targets in SC. Because MCT1 inhibitors such as AZD3965 have entered clinical trials for several types of cancer (NCT01791595), it is expected that patients with refractory SC will also benefit in the future (60). The role of NRs, glucose metabolism, and the microenvironment in tumor initiation and development are presented in Fig. S1.

Immunotherapy, which has recently attracted the most attention in cancer treatment, was developed based on an understanding of the interactions between tumor evasion and microenvironmental changes (6,61). Among cutaneous tumors, immunotherapy using anti-PD-L1 has exhibited the most significant results in malignant melanoma; however, data regarding the efficacy of this treatment in SC are insufficient (62-65). The SP263 assay was selected because it exhibits superiority in many cancer types (66), and counting tumor cells alone was reasonable because most patients with SC present fewer immune cells around the tumor. In this study, the positivity rate of PD-L1 and SP263 was approximately 13% (5/39), including four extraocular tumors. This positivity rate is generally lower than that observed in breast cancer, non-small cell lung cancer, or malignant melanoma (67). This can be explained as follows: First, most SC cases in our cohort were of a limited stage, and second, SC may not be a highly immunogenic tumor. Although no significant relationships were identified between PD-L1 expression and the clinical variables or outcomes, our findings are meaningful because some patients, especially those with extraocular SC, may benefit from anti-PD-L1 treatment.

SC exhibits significant variations in clinical presentation and prognosis depending on its location. Periocular SC is particularly susceptible to diagnostic delays, potential spread into the conjunctiva, and poorer prognosis due to its distinct anatomy. This leads to different approaches in staging, treatment strategies, and surveillance protocols compared to extraocular SC (7). Building on these findings, our current study focused on analyzing whether there are differences in the correlations and expression patterns of various markers based on tumor location. However, as indicated in Table III, our analysis did not reveal any statistically significant differences in the expression of NR, PD-L1, or glucose metabolic markers between the two groups. This lack of significant findings can be attributed to several factors. Firstly, the complexity of the involved molecular pathways may not have been fully captured by the assessed markers. It is possible that other unmeasured molecular factors play a role in differentiating the periocular SC from the extraocular SC, potentially explaining the observed differences in correlation patterns rather than in overall expression levels. Secondly, the initial tumor stages between the two groups varied notably, with the periocular group including a higher proportion of advanced T-category tumors (32%) compared to the extraocular group, where only 6% of the cases were classified as T3 or higher. This disparity in clinical severity may have influenced the expression of these markers, complicating the detection of significant differences between the groups. Given these considerations, we believe that while our study did not find statistically significant differences in marker expression between the periocular and extraocular SC groups, these results should be interpreted with caution. This study focused primarily on the expression of nuclear receptors and glucose metabolic pathway proteins in SC. Although we identified AR and MCT1 as potential biomarkers, we did not perform functional experiments to elucidate the mechanisms by which these proteins influence SC progression. This represents a notable shortcoming, as these functional studies are critical for validating the roles of AR and MCT1 in tumorigenesis. To further explore these mechanisms, several research methods can be suggested as follows: One approach involves using RNA interference (siRNA) or short hairpin RNA (shRNA) to knockdown AR and MCT1 genes to observe their effects on SC cell proliferation, migration, and invasion. Another method would be to overexpress AR and MCT1 by using plasmids or viral vectors, allowing for the evaluation of functional changes in SC cells. Additionally, conducting immunoprecipitation (Co-IP) experiments could be valuable in studying the interactions between AR, MCT1, and other proteins. Finally, using quantitative PCR (qPCR) and Western Blot analyses could help detect changes in gene and protein expression levels following the knockdown or overexpression of AR and MCT1. These approaches will provide a more comprehensive understanding of the molecular mechanisms underlying SC and help to validate the potential of AR and MCT1 as therapeutic targets.

In conclusion, we explored the expression of NRs, PD-L1, and glucose metabolic pathway proteins in SC and found that

low AR and high MCT1 expression were poor prognostic markers. Our results provide a rationale for the use of anti-AR or immune checkpoint inhibitors in patients with advanced SC, particularly in cases where complete surgical resection is not feasible or the tumor has metastasized. Additionally, investigating the crosstalk between NR and metabolic dysregulation in SC will be crucial for developing more effective therapeutic strategies in future.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

Conceptualization and supervision was performed by HC and JEK. Research was performed by YJC, NK and SIK. YJC, MKY, HC and JEK analyzed the data. MKY and YJC confirm the authenticity of all the raw data. YJC wrote the manuscript. Reviewing and editing was performed by MKY, HC and JEK. Funding acquisition was performed by HC. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Seoul National University Hospital (H-1905-059-1032). The requirement for written informed consent was waived due to the retrospective nature of this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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